e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2022; 12(1);01-09
Original Research Article

Prospective, Open Labelled, Randomised, Parallel Group Study To Evaluate The Efficacy And Safety Of Metformin Add- On Therapy To Standard ATT In Newly Diagnosed Pulmonary Tuberculosis Patients

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Received: 01-11-2021 / Revised: 28-11-2021 / Accepted: 22-12-2021
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Conflict of interest: Nil

Abstract

Aim: Clinical Evaluation of Efficacy and Safety of Metformin add- on Therapy to Standard ATT in Newly Diagnosed Pulmonary Tuberculosis Patients

Methods: This was a prospective, open labelled, randomised, parallel group study conducted in KMCH,Katihar, Bihar in Department of Pulmonary Medicine for 1 year. involving 100 tuberculosis patients. Patients were screened and those who fulfilled the selection criteria were included in the study. Patients were randomly allocated to either of the two groups – Control group and Metformin group with 50 patients in each group. In control group, patients received only standard ATT and in Metformin group, patients received Metformin 250 mg twice daily along with standard ATT.

Results: The mean age of the patients in control group was 44 (±11.8) years and in Metformin group, it was 40.3 (±11.1) years. In control group, there were 35 males and 15 females and in Metformin group, 33 males and 17 females. There was no significant difference seen in age and gender distribution of the patients between two groups, as evidenced by the p value more than in unpaired t test for age and chi square test for gender. The average time taken for sputum smear conversion was significantly lower in the Metformin group in comparison with the control group ($p = 0.011$, unpaired t-test). It was about 3.5 (±1.64) weeks in Metformin group while it was 4.8 (±2.21) weeks in the control group. All the subjects enrolled in the study were non-diabetics. At the time of enrollment, their fasting and post prandial blood sugar and HbA1c values were measured and only those who were having normal values were selected for the study. The mean fasting blood sugar was 96.5±8.8mg/dl and 92.2±11.4mg/dl and the mean sugar values at post prandial state was 127.22±24.15 mg/dl and 125.98±30.11 mg/dl in control and Metformin groups respectively at the time of enrollment. In control group, the baseline HbA1c was 4.82±0.41 % and it was 4.95±0.63 % in Metformin group. Adverse events were seen in 5 patients (10%) in control group and 7 patients (14%) in Metformin group. The difference was not statistically significant (p value = 0.81, chi square test). All of the adverse events were only minor in nature and gastrointestinal related problems like nausea, vomiting and gastritis.

Conclusion: It was observed that the average time taken for sputum smear conversion was 3.5 weeks in Metformin group and 4.8 weeks in control group. There were no serious adverse events and most of the adverse events were gastrointestinal related and minor in nature.

Keywords: Metformin, Sputum conversion, TB

Introduction:

Tuberculosis (TB) is a pressing public health problem affecting millions of people globally and remains a major cause of mortality despite the availability of effective drugs and chemotherapy. World Health Organization (WHO) defines TB deaths as patients dying during TB treatment, irrespective of cause.[1] The global mortality in HIV-negative TB was reported to be about 1.2 million in 2019. China is one of the high TB-burden countries. There were an estimated 833,000 TB cases, and an estimated 33,200 fatal cases in 2019.[2]

As a patient diagnosed of TB may not die directly from the disease itself, all-cause mortality was generally used for the description of TB-related deaths. Some studies had reported that human immunodeficiency virus (HIV) and multidrug-resistant and extensively drug-resistant TB (MDR/XDR-TB) attributed to risk factors for TB deaths.[3–5] Other studies reported that malignant comorbidities and non-infectious diseases such as diabetes, renal failure, liver cirrhosis, cardiovascular disease were common risk factors of TB-related death.[6–9] Aging was considered a natural hazard of mortality in many studies.[10] It has been recognized that older people are more vulnerable to developing active tuberculosis and reactivation of latent tuberculosis. But comparison of causes of death in TB patients of different age groups was seldom made.

Metformin is the commonly used anti hyperglycaemic drug, approved by US FDA (United States Food & Drug Administration) in 1995 for the treatment of type 2 Diabetes mellitus. Metformin reduces the hepatic glucose production by inhibiting gluconeogenesis and exerts some action on mitochondrial respiration by reducing the intracellular levels of ATP (Adenosine triphosphate) and increasing AMP (Adenosine monophosphate) levels. It stimulates AMP dependent protein kinase (AMPK) which in turn stimulates fatty acid oxidation, increases glucose uptake, reduces lipogenesis and gluconeogenesis in the liver.[11] The aim of the study was to evaluate the efficacy and safety of Metformin add-on therapy to standard ATT in newly diagnosed pulmonary tuberculosis patients.

Material and methods

This was a prospective, open labelled, randomised, parallel group study conducted in the Department Of Pulmonary Medicine, Katihar Medical College and Hospital, Katihar, Bihar, India for 1 year.involving 100 tuberculosis patients. Patients were screened and those who fulfilled the selection criteria were included in the study.

Methodology

All the patients were explained about the study in detail and informed consent was obtained from all the participants. After enrolment, patients were randomly allocated to either of the two groups-Control group and

Metformin group with 50 patients in each group. The randomisation was simple randomisation applied using computer generated random number tables. In control group, patients received only standard ATT and in Metformin group, patients received Metformin 250 mg twice daily along with standard ATT. Patients with newly diagnosed sputum smear positive pulmonary tuberculosis patients, Patients sensitive to ATT drugs based on GeneXpert analysis were included in this study. Patients with other co-morbid conditions, including diabetes mellitus and hypertension, Pregnant and nursing women were excluded from the study.

Study Medications

In the control group, patients received the standard anti-tubercular treatment (ATT), i.e. Isoniazid (H), Rifampicin (R), Pyrazinamide (Z) and Ethambutol (E) for the first 2 months (intensive phase) followed by Isoniazid (H), Rifampicin (R) and Ethambutol (E) for next 4 months (continuation phase). The drugs were given as fixed dose combinations based on the weight of the patients. The total duration of treatment was 6 months. In the Metformin group, in addition to the standard ATT, Metformin 250 mg was given twice daily, after food, for 6 months. Patients in both the groups were followed up for a period of 6 months.

All the laboratory parameter like complete blood count – Haemoglobin (Hb), total Red Blood Cell (RBC) count, total White Blood Cell (WBC) count, Differential count, platelet count, Erythrocyte Sedimentation Rate (ESR), Liver function tests (LFT) – Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatise (ALP), Total bilirubin and Direct bilirubin, Renal function tests (RFT)-Blood Urea Nitrogen, (BUN) and Creatinine, Fasting Blood Sugar (FBS), Post Prandial Blood, Sugar (PPBS) and glycated haemoglobin (HbA1c), Sputum smear examination, Drug sensitivity testing using GeneXpert,Chest X-ray were studied.

Statistical Analysis

All the variables were subjected to descriptive analysis and summary statistics was generated. Chi square test was used for analyzing drug resistance and sputum conversion. All continuous variables were tested for significant differences by using paired t test within group and unpaired t test between groups. Average duration for sputum conversion between groups was statistically compared by using unpaired t test. Blood sugar values obtained during periodic assessments were analyzed using repeated measures ANOVA within group and one-way ANOVA between the groups.

Results

The mean age of the patients in control group was 44 (±11.8) years and in Metformin group, it was 40.3 (±11.1) years. In control group, there were 35 males and 15 females and in Metformin group, 33 males and 17 females. There was no significant difference seen in age and gender distribution of the patients between two groups, as evidenced by the p value more than in unpaired t test for age and chi square test for gender. Hence, both the groups were comparable in terms of age and gender.

Sputum smear examination was done at baseline and once a week till it became negative. Weekly sputum smear assessment showed that significant number of patients attained smear negativity in the Metformin group compared to the control group. The number of patients who attained sputum smear conversion in both the groups is shown in table 1. In metformin group, one patient remained sputum positive after completion of intensive phase and in control group 10 patients remained sputum positive. The average time taken for sputum smear

conversion was significantly lower in the Metformin group in comparison with the control group (p = 0.011, unpaired t-test). It was about 3.5 (±1.64) weeks in Metformin group while it was 4.8 (±2.21) weeks in the control group.

Table 1: Sputum smear conversion (positive to negative)

Week	Control	Metformin	p-value
1	4 (8%)	7 (14%)	0.10
2	6 (12%)	15 (30%)	0.029
3	12 (24%)	22 (44%)	0.031
4	27 (54%)	39 (78%)	0.011
5	34 (68%)	40 (80%)	0.061
6	39 (78%)	46 (92%)	0.026
7	40 (80%)	49 (98%)	0.01
8	42 (84%)	49 (98%)	0.021

Table 2: Complete Blood Count and Biochemical parameters

	Baseline	Control End	p-value	Baseline	Metformin End	p-value	p value $
	(mean±SD)	(mean±SD)	#	(mean±SD)	(mean±SD)	#	(intergroup)
Hb	12.16±1.08	12.08±0.86	0.52	12.27±1.97	12.45±1.73	0.11	0.08
T.RBC	4.32±0.53	4.35±0.48	0.77	4.30±0.69	4.33±0.60	0.45	0.45
T.BC	8636.96±1239.24	7506.52±847.32	<0.00001*	9216.33±1431.11	7767.35±1090.95	<0.00001*	0.08
N	52.19±5.76	51.71±6.5	0.61	54.88±7.38	54.57±7.17	0.65	0.03
E	2.43±1.08	2.41±0.98	0.30	2.42±1.55	2.38±1.43	0.17	0.41
L	39.78±5.79	40.43±6.55	0.42	39.89±10.31	40.20±7.19	0.25	0.45
M	3.10±1.12	3.15±1.19	0.92	3.78±2.18	3.53±1.90	0.18	0.12
Platelets	2.52±0.78	2.57±0.77	0.22	2.77±0.59	2.80±0.57	0.19	0.06
ESR	57.47±16.39	24.69±7.82	<0.00001*	68.02±16.33	28.28±6.06	<0.00001*	0.41
Renal function tests							
BUN	9.97±3.46	10.06±2.5	0.75	10.02±3.9	10.34±4.19	0.05	0.34
Creatinine	0.79±0.23	0.80±0.22	0.65	0.77±0.24	0.78±0.23	0.17	0.36
Liver function tests							
AST	36.19±5.65	50.30±17.52	<0.00001*	38.08±6.08	51.98±13.84	<0.00001*	0.31
ALT	37.43±6.11	55.69±21.27	<0.00001*	35.76±5.76	57.47±21.23	<0.00001*	0.36
ALP	88.71±15.99	108.26±19.03	<0.00001*	87.94±16.46	110.16±20.52	<0.00001*	0.33
T.Bil	0.72±0.20	0.74±0.22	0.07	0.80±0.29	0.83±0.32	0.44	0.45
D.Bil	0.24±0.10	0.26±0.11	0.05	0.26±0.15	0.28±0.12	0.52	0.21

N-Neutrophils, E-Eosinophils, L-Lymphocytes, M-Monocytes, BUN-Blood Urea Nitrogen, AST- Aspartate transaminase, ALT- Alanine transaminase, ALP- Alkaline phosphatase, T.Bil- Total bilirubin, D.Bil- Direct bilirubin Statistics:# Control group (Baseline vs End) and Metformin group (Baseline vs End)- paired t test $ Control vs Metformin- unpaired t test * p-value <0.05 was considered statistically significant

Drug Resistance Pattern

Drug susceptibility testing was performed at the end of intensive phase for patients who remained sputum positive, in both the groups using GeneXpert. In Metformin group, one patient who remained sputum positive had resistance for Rifampicin. In control group, out of 10 patients who remained sputum positive, 4 patients had resistance for Rifampicin and one patient had indeterminate result in GeneXpert. The sputum of the

patient who had indeterminate result in GeneXpert was analysed in LPA and found to have INH resistance. The other 6 patients in control group who were sputum positive showed sensitivity to the standard ATT and hence they were continued on the same medications and eventually they became sputum negative. The difference in the development of drug resistance between the two groups was not statistically significant (p value=0.35, chi square test). The drug resistant patients were removed from the study and appropriate alternate drug regimens were provided to them.

The blood parameters such as Haemoglobin, total RBC count, total WBC count, Differential count and platelet count were measured at the baseline and at the end of the study. The difference noted between the values observed before and after treatment was not statistically significant between Metformin and control groups. The analysis was done by using unpaired t test (between group analyses) and the p value was more than 0.05. Within group analysis was done by using paired t test which showed that there was a reduction in total WBC count and ESR within control and Metformin groups and the reduction was statistically significant (p-value less than 0.05). The other parameters did not show significant changes in the within group analysis.

Renal function tests which include Blood urea nitrogen (BUN) and serum creatinine did not show any significant differences within the groups and between the groups.

Liver function tests showed significant increase in the liver enzymes- AST, ALT and ALP, at the end of the study when compared with baseline values. The increase was seen in both control and Metformin groups but inter group comparison did not show any statistically significant difference in the enzyme levels. There was no significant difference in the total and direct bilirubin values both within the groups and between the groups.

All the subjects enrolled in the study were non-diabetics. At the time of enrollment, their fasting and post prandial blood sugar and HbA1c values were measured and only those who were having normal values were selected for the study. The mean fasting blood sugar was 96.5±8.8mg/dl and 92.2±11.4mg/dl and the mean sugar values at post prandial state was 127.22±24.15 mg/dl and 125.98±30.11 mg/dl in control and Metformin groups respectively at the time of enrollment. In control group, the baseline HbA1c was 4.82±0.41

% and it was 4.95±0.63 % in Metformin group.

After the initiation of treatment, random blood sugar was measured once in 15 days for first two months and once in a month thereafter. Within group analysis was done using repeated measures ANOVA and between group analysis was done by using one-way ANOVA to detect the differences in random blood sugar values. There was no statistically significant difference noted in the RBS values within the groups in both control and Metformin groups. When RBS values of control and Metformin groups were compared, it showed significant difference between the groups ($p<0.001$). Though statistically significant, there was no clinical significance as the mean values were within the normal range.

Adverse events were seen in 5 patients (10%) in control group and 7 patients (14%) in Metformin group. The difference was not statistically significant (p value = 0.81, chi square test). All of the adverse events were only minor in nature and gastrointestinal related problems like nausea, vomiting and gastritis.

Discussion

Sputum smear examination is the test which is usually done to assess the treatment outcome in pulmonary tuberculosis patients. It is an inexpensive and easy method when compared to sputum culture. Sputum smear examination is usually done at the end of intensive phase and if it becomes negative, it indicates good prognosis. If the sputum smear remains positive despite treatment, it might result in treatment failure, relapse and increase the chance of drug resistance.[12,13] Sputum smear positive patients are highly infectious and one of the important goals of anti-tubercular therapy is to render the patients non-infectious as a smear positive patient can infect more than 10 persons annually.[14]

In our study, the average time taken for smear conversion in control group was 4.8 weeks, which was almost similar to the results obtained from a prospective study done by Parikh et al., in 2012. In that study, the average time required for sputum smear conversion was 5 weeks in patients who were on category I DOTS treatment[19]. In Metformin group, the average time taken for sputum conversion was 3.5 weeks, which was significantly less when compared to control group and Parikh et al.[15]

In this study, Metformin added to standard therapy was found to have significant effect on sputum smear conversion. The number of patients who had become smear negative was significantly high in the Metformin group when compared to control. This difference was observed every week and at the end of 8 weeks, 49 patients (98%) in Metformin group attained smear negativity as against 42 patients (84%) in the control group.

The role of Metformin in tuberculosis has been studied only in diabetic patients so far. Singhal et al., in their study found that tuberculous patients, who were taking Metformin for Diabetes showed reduced number of pulmonary cavities when compared to the patients who were on other anti-diabetic medications.[16] Ye-Jin Lee et al, in their retrospective study found that pulmonary tuberculosis patients with cavitatory TB taking Metformin for Diabetes showed significantly higher sputum culture conversion rates at the end of two months.[17] Y. Ma et al (2018), in their retrospective cohort study involving TB patients with Diabetes, found out that Metformin treatment had a favourable effect on treatment success rate, sputum culture conversion at the end of two months and also the relapse rates when compared to the diabetic patients who were not on Metformin.[18]

In the present study, drug resistance pattern also showed changes between the control and Metformin group. Drug sensitivity testing was done using the molecular methods, GeneXpert and/ or LPA at the end of 2 months. It was observed that 5 patients (10%) in the control group showed drug resistance, 4 patients became resistant to Rifampicin, identified using GeneXpert and 2 patients to Isoniazid, and identified using LPA. In Metformin group, drug resistance was seen in only one patient (2%) who demonstrated resistance for Rifampicin.

One of the reasons for antibiotic resistance in tuberculosis is the formation of persister phenotypes of Mycobacteria which can survive even in the presence of antibiotics. These are slow growing and genetically similar to susceptible bacteria.[19] The main mechanism of persister formation is utilization of the NAD (Nicotinamide adenine dinucleotide) pathway and NDH-I (NADH dehydrogenase-I) for ATP synthesis. NDH-I is similar to human mitochondrial complex-I. Metformin is an inhibitor of mitochondrial complex-I and hence it could also inhibit NDH-I of Mycobacteria and prevent the formation of

persister phenotypes, thereby preventing resistance.

Along with antibiotics, host immune mechanisms are very important in destroying the TB bacilli. In animal models of TB, Metformin treatment increased the production of CD4+ and CD8+ T-lymphocytes and there are also an increased percentage of Interferon-□ secreting CD8+ cells. By inhibiting mitochondrial complex-I, Metformin increases the production of mitochondrial ROS and damages the bacterial cell.[15] Mycobacteria, on entering the host cells by phagocytosis, prevents the maturation of phagosome and starts replicating within the cell. Phagosome maturation is essential for eliminating the pathogen. Autophagy is a defense mechanism which involves the formation of autophagosome, a double membrane vesicle engulfing the cellular components along with the microbes and this autophagosome then fuses with the lysosome, leading to degradation of the cellular components.[20] Metformin was found to induce autophagy and phagolysosome fusion in the host cells.[15]

In the present study, adverse drug reactions were seen in 5 patients (10%) in the control group and 7 patients (14%) in the Metformin group and the difference noted between the groups was not statistically significant. The adverse reactions seen in both the groups were only mild and most of them were gastrointestinal related symptoms like nausea, vomiting and gastritis. These adverse events are not specific to Metformin and could occur with anti TB drugs also. Hypoglycaemia was not reported in any of the patients in the Metformin group.

When the incidence of adverse events in this study was compared to literature reported data, it was noted that the occurrence of adverse events in the present study was less. Singh A et al. (2015), in their review article, stated that the incidence of adverse events in patients taking ATT was between 2.3% to 17%, commonly involving hepatobiliary and gastrointestinal system. The authors of the review article further quoted an Indian study done by Shinde et al., which reported the incidence of GI adverse effects like nausea, vomiting and abdominal pain as 12.5% with ATT.[21] Bray GA et al., in their article, reported that gastrointestinal adverse effects were common with Metformin and the incidence was around 28%.[22] However in the present study, the incidence of adverse events was less, 10% in control group and 14% in metformin group.

Conclusion

It was observed that the average time taken for sputum smear conversion was 3.5 weeks in Metformin group and 4.8 weeks in control group. There were no serious adverse events and most of the adverse events were gastrointestinal related and minor in nature.

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